Product Description
SALSA® MLPA® Probemix P211-B5 HSP region

To be used with the MLPA General Protocol.

Version B5
For complete product history see page 8.

Catalogue numbers
- **P211-025R:** SALSA® MLPA® Probemix P211 HSP region, 25 reactions
- **P211-050R:** SALSA® MLPA® Probemix P211 HSP region, 50 reactions
- **P211-100R:** SALSA® MLPA® Probemix P211 HSP region, 100 reactions

SALSA® MLPA® Probemix P211 HSP region (hereafter: P211 HSP region) is to be used in combination with:

1. SALSA® MLPA® Reagent Kit (Cat. No: EK1-FAM, EK1-CY5, EK5-FAM, EK5-CY5, EK20-FAM),
2. Data analysis software Coffalyser.Net™ (Cat. No: n.a.)

Volumes and ingredients

Volumes			Ingredients
P211-025R	**P211-050R**	**P211-100R**	
40 µl	80 µl	160 µl	Synthetic oligonucleotides, oligonucleotides purified from bacteria, Tris-HCl, EDTA

The MLPA probemix is not known to contain any harmful agents. Based on the concentrations present, none of the ingredients are hazardous as defined by the Hazard Communication Standard. A Safety Data Sheet (SDS) is not required for this product: none of the ingredients contain dangerous substances at concentrations requiring distribution of an SDS (as per Regulation (EC) No 1272/2008 [EU-GHS/CLP] and 1907/2006 [REACH] and amendments).

Storage and handling

Recommended storage conditions		

A shelf life of until the expiry date is guaranteed, when stored in the original packaging under recommended conditions. For the exact expiry date, see the label on the vial. This product should not be exposed to more than 25 freeze-thaw cycles. Do not use the product if the packaging is damaged or opened. Leave chemicals in original containers. Waste material must be disposed of in accordance with the national and local regulations.

Certificate of Analysis
Information regarding quality tests and a sample electropherogram from the current sales lot is available at www.mrcholland.com.

Precautions and warnings
For professional use only. Always consult the most recent product description AND the MLPA General Protocol before use: www.mrcholland.com. It is the responsibility of the user to be aware of the latest scientific knowledge of the application before drawing any conclusions from findings generated with this product.

General information
SALSA® MLPA® Probemix P211 HSP region is a **research use only (RUO)** assay for the detection of deletions or duplications in the *SPAST* gene and other genes located in the 2p22.3 region and 15q11.2 region, which are associated with Hereditary spastic paraplegias (HSPs).

HSPs are a large and diverse group of genetically heterogeneous neurodegenerative disorders characterised by progressive lower limb/lower extremity spasticity and weakness. Defects in the *SPAST* gene (also known

as *SPG4*) on chromosome 2p22 are identified as one of the causes for autosomal dominant pure HSP. The protein encoded by this gene is spastin which is involved in membrane shaping and modelling events.

This probemix contains probes for the first exon and intron and probes for exon 16 and 17 of the *SPAST* gene and the flanking regions of *SPAST* (2p22.3 region). This probemix furthermore contains probes for the *NIPA1* gene (also known as *SPG6*) and probes for the genes nearby *NIPA1*, such as the *WHAMML1* and *HERC2P2* genes (15q11.2 region). Probes for these flanking genes of *NIPA1* have been included to distinguish *NIPA1* defects from larger deletions in the Prader-Willi/Angelman syndrome region. The database of genomic variants mentions several copy number changes in this genomic region that have been found in healthy individuals (see https://dgv.tcag.ca/dgv/app/home).

This product is not CE/FDA registered for use in diagnostic procedures. The SALSA® MLPA® technique is covered by US patent 6,955,901 and corresponding patents outside the US. The purchase of this product includes a license to use only this amount of product solely for the purchaser's own use.

Gene structure and transcript variants:
Entrez Gene shows transcript variants of each gene: https://www.ncbi.nlm.nih.gov/gene
For NM_ mRNA reference sequences: https://www.ncbi.nlm.nih.gov/nuccore?db=nucleotide
Locus Reference Genomic (LRG) database: https://www.lrg-sequence.org/
Matched Annotation from NCBI and EMBL-EBI (MANE): https://www.ncbi.nlm.nih.gov/refseq/MANE

Exon numbering
The *SPAST* exon numbering used in this P211-B5 HSP region product description is the exon numbering derived from MANE project (release version 1.0) based on MANE Select transcript NM_014946.4. The exon numbering used in previous versions of this product description can be found in between brackets in Table 2. As changes to the databases can occur after release of this product description, the NM_ sequence and exon numbering may not be up-to-date.

Probemix content
P211-B5 HSP region contains 39 MLPA probes with amplification products between 128 and 462 nucleotides (nt). This includes 13 probes for the 2p22.3 region, including probes for the *SPAST* gene. This probemix furthermore contains 13 probes for the 15q11.2 region, which encompasses the NIPA1, NIPA2, WHAMML1, and HERC2P2 genes. In addition, 13 reference probes are included that detect autosomal chromosomal locations. Partial probe sequences and the identity of the genes detected by the reference probes are available online (www.mrcholland.com).

This probemix contains nine quality control fragments generating amplification products between 64 and 105 nt: four DNA Quantity fragments (Q-fragments), two DNA Denaturation fragments (D-fragments), one Benchmark fragment, and one chromosome X and one chromosome Y-specific fragment (see table below). More information on how to interpret observations on these control fragments can be found in the MLPA General Protocol and online at www.mrcholland.com.

Length (nt)	Name
64-70-76-82	Q-fragments (only visible with <100 ng sample DNA)
88-96	D-fragments (low signal indicates incomplete denaturation)
92	Benchmark fragment
100	X-fragment (X chromosome specific)
105	Y-fragment (Y chromosome specific)

MLPA technique
The principles of MLPA (Schouten et al. 2002) are described in the MLPA General Protocol (www.mrcholland.com).

MLPA technique validation
Internal validation using 16 different DNA samples from healthy individuals is required, in particular when using MLPA for the first time, or when changing the sample type or the sample handling procedure, DNA extraction method or instruments used. This validation experiment should result in a standard deviation ≤0.10 for all reference probes over the experiment.

Required specimens
Extracted DNA, free from impurities known to affect MLPA reactions. MRC Holland has tested and can recommend the following extraction methods:

- QIAGEN Autopure LS (automated) and QIAamp DNA mini/midi/maxi kit (manual)
- Promega Wizard Genomic DNA Purification Kit (manual)
- Salting out (manual)

All samples tested, including reference DNA samples, should be derived from the same tissue type, handled using the same procedure, and prepared using the same DNA extraction method when possible. For more information please refer to the section on DNA sample treatment found in the MLPA General Protocol.

Reference samples
A sufficient number (≥3) of different reference samples from unrelated individuals should be included in each MLPA experiment for data normalisation. Reference samples should be derived from individuals who are from families without a history of Hereditary spastic paraplegias. More information regarding the selection and use of reference samples can be found in the MLPA General Protocol (www.mrcholland.com).

Positive control DNA samples
MRC Holland cannot provide positive DNA samples. Inclusion of a positive sample in each experiment is recommended. Coriell Institute (https://catalog.coriell.org) and Leibniz Institute DSMZ (https://www.dsmz.de) have diverse collections of biological resources which may be used as positive control DNA samples in your MLPA experiments. The quality of cell lines can change; therefore samples should be validated before use.

Data analysis
Coffalyser.Net should be used for data analysis in combination with the appropriate lot-specific Coffalyser sheet. For both, the latest version should be used. Coffalyser.Net is freely downloadable at www.mrcholland.com. Use of other non-proprietary software may lead to inconclusive or false results. For more details on MLPA quality control and data analysis, including normalisation, see the Coffalyser.Net Reference Manual.

Interpretation of results
The standard deviation of each individual reference probe over all the reference samples should be ≤0.10 and the final ratio (FR) of each individual reference probe in the patient samples should be between 0.80 and 1.20. When these criteria are fulfilled, the following cut-off values for the FR of the probes can be used to interpret MLPA results for autosomal chromosomes or pseudo-autosomal regions:

Copy number status	Final ratio (FR)
Normal	0.80 < FR < 1.20
Homozygous deletion	FR = 0
Heterozygous deletion	0.40 < FR < 0.65
Heterozygous duplication/gain	1.30 < FR < 1.65
Heterozygous triplication/homozygous duplication/gain	1.75 < FR < 2.15
Ambiguous copy number	All other values

Note: The term “dosage quotient”, used in older product description versions, has been replaced by “final ratio” to become consistent with the terminology of Coffalyser.Net (Calculations, cut-offs and interpretation remain unchanged.) Please note that Coffalyser.Net also shows arbitrary borders as part of the statistical analysis of

results obtained in an experiment. As such, arbitrary borders are different from the final ratio cut-off values shown here above.

- Arranging probes according to chromosomal location facilitates interpretation of the results and may reveal more subtle changes such as those observed in mosaic cases. Analysis of parental samples may be necessary for correct interpretation of complex results.
- False positive results: Please note that abnormalities detected by a single probe (or multiple consecutive probes) still have a considerable chance of being a false positive result. Sequence changes (e.g. single nucleotide variants (SNVs), point mutations) in the target sequence detected by a probe can be one cause. Incomplete DNA denaturation (e.g. due to salt contamination in the DNA sample) can also lead to a decreased probe signal, in particular for probes located in or near a GC-rich region. The use of an additional purification step or an alternative DNA extraction method may resolve such cases. Additionally, contamination of DNA samples with cDNA or PCR amplicons of individual exons can lead to an increased probe signal (Varga et al. 2012). Analysis of an independently collected secondary DNA sample can exclude these kinds of contamination artefacts.
- Normal copy number variation in healthy individuals is described in the database of genomic variants: https://dgv.tcag.ca/dgv/app/home. Users should always consult the latest update of the database and scientific literature when interpreting their findings.
- Not all abnormalities detected by MLPA are pathogenic. In some genes, intragenic deletions are known that result in very mild or no disease (as described for *DMD* by Schwartz et al. 2007). For many genes, more than one transcript variant exists. Copy number changes of exons that are not present in all transcript variants may not have clinical significance. Duplications that include the first or last exon of a gene (e.g. exons 1-3) might not result in inactivation of that gene copy.
- Copy number changes detected by reference probes or flanking probes are unlikely to have any relation to the condition tested for.
- False results can be obtained if one or more peaks are off-scale. For example, a duplication of one or more exons can be obscured when peaks are off-scale, resulting in a false negative result. The risk on off-scale peaks is higher when probemixes are used that contain a relatively low number of probes. Coffalyser.Net software warns for off-scale peaks while other software does not. If one or more peaks are off-scale, rerun the PCR products using either: a lower injection voltage or a shorter injection time, or a reduced amount of sample by diluting PCR products.

Limitations of the procedure

- In most populations, the major cause of genetic defects in the *SPAST* gene are small (point) mutations, none of which will be detected by using P211 HSP region.
- MLPA cannot detect any changes that lie outside the target sequence of the probes and will not detect copy number neutral inversions or translocations. Even when MLPA did not detect any aberrations, the possibility remains that biological changes in that gene or chromosomal region *do* exist but remain undetected.
- Sequence changes (e.g. SNVs, point mutations) in the target sequence detected by a probe can cause false positive results. Mutations/SNVs (even when >20 nt from the probe ligation site) can reduce the probe signal by preventing ligation of the probe oligonucleotides or by destabilising the binding of a probe oligonucleotide to the sample DNA.

Confirmation of results

Copy number changes detected by only a single probe always require confirmation by another method. An apparent deletion detected by a single probe can be due to e.g. a mutation/polymorphism that prevents ligation or destabilises the binding of probe oligonucleotides to the DNA sample. Sequence analysis can establish whether mutations or polymorphisms are present in the probe target sequence. The finding of a heterozygous mutation or polymorphism in sequence data indicates that two different alleles of the sequence are present in the sample DNA and that a false positive MLPA result was obtained.

Copy number changes detected by more than one consecutive probe should be confirmed by another independent technique such as long range PCR, qPCR, array CGH or Southern blotting, whenever possible. Deletions/duplications of more than 50 kb in length can often be confirmed by FISH.

***SPAST* mutation database**

https://databases.lovd.nl/shared/genes/SPAST We strongly encourage users to deposit positive results in the Leiden Open Variation Database (LOVD). Recommendations for the nomenclature to describe deletions/duplications of one or more exons can be found on https://varnomen.hgvs.org.

Please report copy number changes detected by the reference probes, false positive results due to SNVs and unusual results to MRC Holland: info@mrcholland.com.

Table 1. P211-B5 HSP region

Length (nt)	MLPA probe	Chromosomal position (hg18)[a]		
		Reference	2p22.3 region	15q11.2 region
64-105	Control fragments – see table in probemix content section for more information			
128	Reference probe 00797-L00093	5q31		
133	**HERC2P2 probe** 09878-L22208			**Exon 23**
139	**SPAST probe** 05659-L22209		**Exon 1**	
148 *	Reference probe 04445-L03831	4q13		
154	**GOLGA6L2 probe** 09873-L10285			**Exon 7**
166 *	Reference probe 16058-L18232	9p21		
173	**SPAST probe** 07128-L06737		**Exon 17**	
179 «	**WHAMML1 probe** 09875-L12280			**Exon 2**
184	Reference probe 02312-L01803	19p13		
190	**SPAST probe** 08262-L08125		**Upstream**	
196 Ø	**SPAST probe** 08263-L08126		**Intron 1**	
202	**NIPA2 probe** 07123-L05171			**Exon 6**
214	**NIPA2 probe** 05767-L05718			**Exon 3**
220	Reference probe 02947-L02379	7q31		
226 Ø	**SPAST probe** 08264-L08127		**Intron 1**	
235	**SPAST probe** 05265-L04648		**Exon 1**	
241	**SPAST probe** 05658-L05111		**Exon 17**	
256	Reference probe 06007-L05432	2q36		
265	**NIPA1 probe** 07124-L05206			**Exon 5**
274	Reference probe 05360-L04739	11p13		
292	Reference probe 08790-L11322	10q21		
299	**SPAST probe** 07127-L06736		**Upstream**	
314	**GOLGA6L2 probe** 09874-L12277			**Exon 8**
320 « ¬	**DPY30 probe** 07130-L12278		**Exon 5**	
328 *	Reference probe 16583-L18726	12q24		
337 ¬	**SLC30A6 probe** 07132-L06741		**Exon 12**	
346	**TUBGCP5 probe** 01321-L12279			**Exon 8**
355 ¬	**DPY30 probe** 07129-L06738		**Exon 2**	
364	Reference probe 10675-L11257	6p12		
382	**CYFIP1 probe** 01818-L01317			**Exon 23**
391	**NIPA1 probe** 07125-L05755			**Exon 3**
400	Reference probe 05343-L04730	1p21		
409	**MAGEL2 probe** 11155-L11839			**Exon 1**
418	**NIPA2 probe** 05117-L04501			**Exon 8**
428	**HERC2P2 probe** 09877-L11168			**Exon 13**
436	**SPAST probe** 08261-L22286		**Upstream**	
445	**SPAST probe** 20720-L28598		**Exon 16**	
454	Reference probe 01051-L00620	8q21		
462	Reference probe 18948-L01619	13q13		

[a] See section Exon numbering on page 2 for more information.
* New in version B5.
« Probe located in or near a GC-rich region. A low signal can be caused by salt contamination in the DNA sample leading to incomplete DNA denaturation, especially of GC-rich regions.
¬ Flanking probe. Included to help determine the extent of a deletion/duplication. Copy number alterations of only the flanking or reference probes are unlikely to be related to the condition tested.
Ø Intron probe. Only included to help determine the extent of a deletion/duplication. Copy number alterations of only this probe are of unknown clinical significance.

The probe lengths in the table above may vary slightly depending on the capillary electrophoresis machine settings. Please see the most up-to-date Coffalyser sheet for exact probe lengths obtained at MRC Holland.

SNVs located in the target sequence of a probe can influence probe hybridisation and/or probe ligation. Single probe aberration(s) must be confirmed by another method.

Table 2. Target and flanking probes arranged according to chromosomal location

Table 2a. 2p22.3 region

Length (nt)	MLPA probe	*SPAST* exon[a]	Ligation site[b] NM_014946.4	Partial sequence[c] (24 nt adjacent to ligation site)	Distance to next probe
320 ¬	07130-L12278	*DPY30* Exon 5	NM_032574.4; 647-648.	TTTTGGATGTAT-AAGAACCTTCCG	15.4 kb
355 ¬	07129-L06738	*DPY30* Exon 2	NM_032574.4; 2 nt before exon 2.	TCTTGTTTTCCA-AGACTGGTATCC	22.8 kb
		start codon	*277-279 (Exon 1)*		
436	08261-L22286	Upstream	1349 nt before exon 1	TGTGGAGATTCT-TGGAAGCTGGAA	0.5 kb
190	08262-L08125	Upstream	881 nt before exon 1	TCATTAGAATAC-AGGGAGCAGAGA	0.7 kb
299	07127-L06736	Upstream (Exon 1)	123 nt before exon 1	AACTGCACATTG-GGAACTGTAGTT	0.4 kb
139	05659-L22209	Exon 1	281-282	GCTGTGAATGAA-TTCTCCGGGTGG	0.1 kb
235	05265-L04648	Exon 1	433-434	ACCTGTACTATT-TCTCCTACCCGC	1.7 kb
196 Ø	08263-L08126	Intron 1	1410 nt after exon 1	CCTCTGCCAAAA-ACACCCACTTTT	14.9 kb
226 Ø	08264-L08127	Intron 1	6932 nt before exon 2	GTCATAGAGTTA-AAAGAGAAATGT	66.7 kb
445	20720-L28598	Exon 16	1976-1977	ACTAAAACCAGA-ACAGGTGAAGAA	7.2 kb
241	05658-L05111	Exon 17	2086-2087	CTTTAGAAGCGT-ACATACGTTGGA	1.9 kb
173	07128-L06737	Exon 17	4016-4017	TAGCCATAAGGT-AAATCATGTCTC	48.2 kb
		stop codon	*2125-2127 (Exon 17)*		
337 ¬	07132-L06741	*SLC30A6* Exon 12	NM_017964.5; 741-742	TGCTATAGCTAT-TGCCTTGATGAC	

Table 2b. 15q11.2 region

Length (nt)	MLPA probe	Gene/exon[a]	Ligation site[b]	Partial sequence[c] (24 nt adjacent to ligation site)	Distance to next probe
346	01321-L12279	*TUBGCP5* Exon 8	NM_052903.6; 793-794.	CAGTGATCCATT-GTATGTTCCAGA	122.3 kb
382	01818-L01317	*CYFIP1* Exon 23	NM_014608.5; 2586-2587	CTGTTGGAAATC-AACCGCATGACC	36.4 kb
418	05117-L04501	*NIPA2* Exon 8 (10)	NM_030922.7; 2318-2319	TGAGCATTCGAT-GGCCTTAGCACC	8.9 kb
202	07123-L05171	*NIPA2* Exon 6 (8)	NM_030922.7; 829-830	GTGGCCAACTTC-GCTGCGTATGCG	13.3 kb
214	05767-L05718	*NIPA2* Exon 3 (4)	NM_030922.7; 589-590	TCTGAGAATAGT-GAAGCAACTCAT	21.4 kb
265	07124-L05206	*NIPA1* Exon 5	NM_144599.5; 615-616	GCTGGGCAGTTT-CACCGTGCCTTC	11.7 kb
391	07125-L05755	*NIPA1* Exon 3	NM_144599.5; 260-261	GCCAGATTGGAA-ACTTCCTGGCTT	144.3 kb
179 #	09875-L12280	*WHAMML1* Exon 2	NR_003521.1; 612-613	TTCAAGGACACC-GAAAAGCCAACA	106.4 kb
133 #	09878-L22208	*HERC2P2* Exon 23	NR_002824.3; 3506-3507	GCCCAGTTAGAT-GACTACTTCCCT	15.1 kb
428 #	09877-L11168	*HERC2P2* Exon 13	NR_002824.3; 1780-1781	TACTGTTACAGA-TGTTCACAAATA	358.1 kb
314 #	09874-L12277	*GOLGA6L2* Exon 8	NM_001304388.1; 2976-2977	AAGAGAAAGATG-AAGATCATCAAT	2.2 kb
154 #	09873-L10285	*GOLGA6L2* Exon 7	NM_001304388.1; 735-736	AGGAGCTGAAGA-AGAAAAATGCCG	202.1 kb
409	11155-L11839	*MAGEL2* Exon 1	NM_019066.5; 3713-3714	AGCAAGATGCTT-GTCCTGAGGTTT	

[a] See section Exon numbering on page 2 for more information.

[b] Ligation sites are relative to the start of the NM_ sequence, and not relative to the coding sequence.
[c] Complete probe sequences are available at www.mrcholland.com. Please notify us of any mistakes: info@mrcholland.com.

¬ Flanking probe. Included to help determine the extent of a deletion/duplication. Copy number alterations of only the flanking or reference probes are unlikely to be related to the condition tested.
Ø Intron probe. Only included to help determine the extent of a deletion/duplication. Copy number alterations of only this probe are of unknown clinical significance.
This probe's specificity relies on a single nucleotide difference compared to a related gene, pseudogene or highly similar region. As a result, an apparent duplication of only this probe can be the result of a non-significant single nucleotide sequence change in the related gene, pseudogene or highly similar region.

SNVs located in the target sequence of a probe can influence probe hybridisation and/or probe ligation. Single probe aberration(s) must be confirmed by another method.

Related products
For related products, see the product page on our website.

References

- Schouten JP et al. (2002). Relative quantification of 40 nucleic acid sequences by multiplex ligation-dependent probe amplification. *Nucleic Acids Res.* 30:e57.
- Schwartz M et al. (2007). Deletion of exon 16 of the dystrophin gene is not associated with disease. *Hum Mutat.* 28:205.
- Varga RE et al. (2012). MLPA-based evidence for sequence gain: pitfalls in confirmation and necessity for exclusion of false positives. *Anal Biochem.* 421:799-801.

Selected publications using P211 HSP region

- De Leva MF et al. (2010). Complex phenotype in an Italian family with a novel mutation in SPG3A. *J. Neurol.*, *257*(3), 328-331.
- Dong EL et al. (2018). Clinical spectrum and genetic landscape for hereditary spastic paraplegias in China. *Mol. Neurodegener. 13*(1), 36.
- Mészárosová AU et al. (2016). SPAST mutation spectrum and familial occurrence among Czech patients with pure hereditary spastic paraplegia. *J. Hum. Genet.*, *61*(10), 845.

P211 product history	
Version	*Modification*
B5	Three reference probes have been replaced.
B4	Four reference probes have been replaced, five flanking probes have been removed and one length has been adjusted.
B3	Three reference probes have been replaced, one removed and the 88 and 96 nt control fragments have been replaced (QDX2).
B2	Seven probes in the 15q11 region between the *NIPA* genes and *MKRN3* have been added. Two DD (DNA Denaturation) control fragments at 88 and 96 nt have been added.
A1	First release.

Implemented changes in the product description
Version B5-02 – 04 November 2025 (05P) - Product description adapted to a new template. - Removed Related SALSA MLPA products section. - Gene structure and transcript variants section: link to MANE website added. - NM sequence of the SPAST, NIPA2, NIPA1 and MAGEL2 probes updated. - Table 1 and 2: Exon numbering/description of the SPAST and NIPA2 probes changed.

More information: www.mrcholland.com; www.mrcholland.eu	
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